

ART. XXX.—*The Biochemical Significance of
Phosphorus.*

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The existence of plant life on the earth is essential for the existence of animal life, since plants alone have the power of living entirely on inorganic materials and of building them into the organic substances necessary for the life of animals. Plant life being thus essential, the chemical necessities of plants will determine the chemical necessities of animals.

Of the long list of known chemical elements only comparatively few are of any biological significance. The most important of those which are absolutely essential for plant and therefore for animal life can be put into one or other of two groups.

1. The Water-borne or Soil elements, present in soil or dissolved in rivers or sea-water. To this group belong sodium, potassium, magnesium, calcium, iron, chlorine, sulphur and phosphorus.
2. The Air-borne elements, present in air and in the case of oxygen and nitrogen usually in the free state. To this group belong oxygen, hydrogen, nitrogen and carbon. Since these elements are air-borne there will never be a serious deficiency of them except in rainless areas. On the other hand there is the possibility of deficiency in the soil elements.

Of the soil elements special interest is attached to phosphorus, not only on account of its presence in practically all tissues both animal and vegetable, but also on account of the fact that of all the elements necessary to life it is the one in obtaining which difficulty may be encountered, since in order to be used by the plant it must be in a form available to the

plant. It is an element vastly important in every department of biology, since the nucleoproteins which constitute so large a part of the nucleus of every living cell are rich in phosphorus, and it is also an essential ingredient of lipoid, which recent research has shown to be so important a component of all living tissue. Lilienfeld and Monti discovered phosphorus in bacteria, and analyses of dry bacteria have shown the percentage of P_2O_5 to be greater than that of any other ash constituent (1). For example, in 100 parts of dry substance of *Bacillus prodigiosus* the total ash = 13.47, K_2O = 1.55, Na_2O = 3.93, CaO = .56, MgO = 1.05, P_2O_5 = 5.12, Cl = .66, $NaCl$ = 1.08, SiO_2 = .07. In 1796 Westrumb demonstrated presence of phosphoric acid in the beer yeast, almost half of the pure ash being phosphoric acid. Yeast placed in a sugar solution bereft of all salts can not bring forth fermentation to any appreciable degree. Mayer placed phosphoric acid in the list of the salts necessary for growth of the yeast plant.

It is a significant fact that all tissues provided for the maintenance of the young are rich in phosphorus. Thus not only eggs and milk, but plant seeds, are rich in this constituent, the percentage of P_2O_5 in the ash of ripe seeds is seldom below 25, and may reach 50 (2). [See analyses of Wolff, Kellner, Lehmann, Presse and Stansell, etc.] Where the phosphorus poor tegument is included in the analysis the results are of course lower. Most of the phosphorus in seeds is in organic combination, such as glycerophosphoric acid or phytovittelinin. The phosphoric acid content in the ash of the underground food reservoirs of the plant is usually markedly lower than in seeds, viz., about 15 per cent., but is very variable. The supply of phosphoric acid has a noteworthy effect on the storing of sugar in storage roots, a rich supply of phosphoric acid causing an increased sugar content in the root (3). Also in the wood of trees, in the sap wood more than in the heartwood, and more in the upper actively growing parts of the tree than in the lower parts; in the bark and in the leaves we find phosphoric acid, in various kinds of combination, as a necessary constituent.

Of essentially animal tissues it forms, in combination with calcium and magnesium, as phosphate, some 85 per cent. of the

bone ash. In muscle the predominating salt is potassium phosphate, the percentage of P_2O_5 in the ash being roughly 35. Brain and nervous tissue are rich in P_2O_5 , the ash containing nearly 35 per cent. In lungs and bronchi again the predominating constituent of the ash is phosphoric acid, obtained for the most part from organic combinations (4). The Liver, Spleen, Thymus, also, being rich in cellular elements, have a high percentage of phosphorus in organic combination.

From the time of Liebig attention has been centred on nitrogen as the food element of living things most liable to fluctuation or deficiency; but it must be remembered that nitrogen is in reality air-borne, and an increased demand for this element can be met by increasing the facilities for trapping the air nitrogen. With phosphorus, however, the case is different. It is a constituent of soil or is water-borne, and may be described as the one element about which plants are conservative. Thus it has been shown that the leaves of deciduous trees before being allowed to fall from the plant have practically all the phosphorus extracted from them (5). In much the same way we find animals to be conservative of this element, so that in the young animal where much phosphorus is needed for the growing skeleton the amount excreted is very low. The same is the case during the processes of dentition and lactation.

The widespread distribution of this element in all living things makes it desirable that there should be in soils a large quantity available to plants. That soils may contain a low percentage of available phosphorus is unfortunately well exemplified in many regions of Australia. This lack of phosphorus has been generally recognised for some time, and is indirectly proved by the excellent results following the use of phosphatic fertilisers. Analyses by agricultural experts in the State of Victoria may be found in the *Agricultural Journal of Victoria*, 1907, comparing the Victorian clay soils with American clay soils. The clay soils are given because they contain a higher percentage of phosphorus than other soils. Thus giving the phosphorus in terms of phosphoric acid, in 100,000 parts of Victorian clay soil we find only 63 parts of phosphoric acid of against 207 parts of phosphoric acid in the same amount of American clay soil. The difference in the sub-soils is not quite

so great, but sufficiently striking to be evident at once; thus per 100,000 parts of clay sub-soil we find in Victoria 66 parts of phosphoric acid as against 159 parts in America. The amount in Mallee soil is very low, namely, 47 parts per 100,000; and American and European authorities look upon 50 parts per 100,000 as the limit below which it is unprofitable to work the soil.

The objects of the following research were:—

1. To ascertain whether plants could accommodate themselves to a soil containing less than the average amount of available phosphorus.
2. To compare the phosphorus content of some typical Australian grown foods with the determinations of phosphorus content in the same kinds of foods in other countries.
3. To determine the distribution and evolutionary significance of phosphorus in muscle, brain, exo- and endo-skeleton of animals.
4. To obtain some quantitative data regarding the leakage of phosphorus from Victorian soils.

Methods.

In all the estimations of phosphorus, except where specially mentioned, the method used was a modification of the Neumann method of phosphorus estimation (6), and was as follows:—The substance, weighed in the fresh and then in the dry state, was ashed. The ash weighed and dissolved in a small quantity of nitric acid, diluted, precipitated with 10 per cent. ammonium molybdate in presence of ammonium nitrate, the quantities of these reagents being used in amounts such as are best calculated to prevent precipitation of any molybdic acid, thus when about 10 milligrams of phosphoric anhydride were roughly expected to be present, 4–5 c.c. of strong nitric were used to dissolve the ash, from 150–200 c.c. of water used to dilute, 85 c.c. of 50 per cent. ammonium nitrate added, and the solution then brought almost to boiling point, 50 c.c. of hot 10 per cent. ammonium molybdate were then added, and the solution kept in motion for one minute, then allowed to

stand 15 minutes, when it was then quickly filtered through a Gooch filter, the collected precipitate washed thoroughly with cold water (about six washings), and then dissolved in a known volume of standard sodium hydrate, boiled to expel all the ammonia thus liberated, and when cool the excess of NaOH back titrated with standard acid. The amount of P_2O_5 calculated from the number of c.c. of the standard alkali used.

Experimental.

A (1).—EXPERIMENTS WITH FODDER GRASSES.

To Professor Ewart I am indebted for his kindness in supplying me with samples of 20 fodder grasses; some of them native to Australia, and some of them introduced, but all acclimatised and growing in a wild state free from any artificial manuring.

It was expected that the native grasses accustomed for a long epoch of time to a low supply of phosphorus in the soil would manage with very little of this element, and therefore an analysis would show a low percentage of P_2O_5 . On the other hand it was expected that the introduced grasses, accustomed to a fairly high supply of phosphorus, would not show the economy practised by the native grasses with regard to this element, but would show on analysis a higher percentage of P_2O_5 , which, however, might be lower than that obtained for the same grasses growing in countries whose soil is not so low in this element. The following table shows the results obtained:—

PERCENTAGE OF P_2O_5 IN FODDERS (DRIED AT 100° C.).
NATIVE AND INTRODUCED GRASS.

		<i>Native.</i>		%	Mean %
Name of Grass					
Imperata arundinacea—Lang Lang Grass	(1)	0.1659	0.1680		
	(2)	0.1701			
Stipa scabra—Rough Spear Grass - -	(1)	0.1600	0.1543		
	(2)	0.1487			
Chloris truncata—Windmill Grass - -	(1)	0.1977	0.2023		
	(2)	0.2069			

Name of Grass		P_2O_5	Mean P_2O_5
Cynodon dactylon—Couch or Doub Grass	(1)	0.1539	0.1609
	(2)	0.1680	
Microlaena stipoides—Weeping Grass	(1)	0.1391	0.1330
	(2)	0.1271	
Panicum effusum—Hairy Millet Grass	(1)	0.0973	0.0988
	(2)	0.1004	
Eragostis pilosa—Lesser Love Grass	(1)	0.2790	0.2656
	(2)	0.2522	
Danthonia penicillata—Wallaby Grass	(1)	0.1348	0.1429
	(2)	0.1511	
Poa caespitosa—Tussock Grass	(1)	0.1311	0.1350
	(2)	0.1390	
Sporobolus indicus—Ratstail Grass	(1)	0.1480	0.1484
	(2)	0.1489	
Anthistira ciliata—Kangaroo Grass	(1)	0.1258	0.1258
Arundo phragmites—Reed Grass	(1)	0.2996	0.2994
	(2)	0.2992	

Introduced.

Oryzopsis miliacea—Rice Millet	(1)	0.3533	0.3880
	(2)	0.4227	
Ehrharta panicea—Panic-like Ehrharta	(1)	0.3775	0.4124
	(2)	0.4473	
Lolium perenne—Perennial Rye Grass	(1)	0.3020	0.3160
	(2)	0.3300	
Bromus unioloides—Prairie Grass	(1)	0.3646	0.3804
	(2)	0.3962	
Holcus lanatus—Yorkshire Fog Grass	(1)	0.1427	0.1463
	(2)	0.1500	
Agropyrum repens—English Couch or	(1)	0.1732	0.1608
Quitch Grass	(2)	0.1484	
Stenotaphrum Americanum—Buffalo Grass	(1)	0.2791	0.2791
Paspalum distichum—Silt Grass	(1)	0.4181	0.4181

The table shows all the native grasses taken, with perhaps the exception of Arundo Phragmites, to have a low percentage of P_2O_5 , and all the introduced, with 2 exceptions, viz., Holcus

lanatus and *Agropyrum repens*, to have a much higher percentage. Of the two exceptions in the list of introduced grasses, it may be said that they can hardly be regarded as fodders at all. They are exceedingly poor grasses whether grown in their native home or in this country, and are frequently regarded as weeds, and weeded out as such. Of the native grass *Arundo phragmites* it may be noted that it always grows in very wet ground, on the edges of rivers, pools or marshes, which fact may possibly account for its greater percentage of phosphorus, since the tendency of the water, especially if impregnated with CO_2 , would be to dissolve phosphate from a considerable depth of soil, and bring it by diffusion into the neighbourhood of the roots, i.e., the water would probably tend to give a larger available supply of P_2O_5 than could be obtained by the dry medium plants.

No record of English or Continental analyses of the particular grasses occurring in the above table could be found; but Balland (7) gives the maximum percentage of P_2O_5 in the fresh millet as .8, which percentage would of course be higher calculated per dry substance, and Wolff (8) in dry millet gives 3.43 per cent. total ash, and in the ash 21.92 per cent. P_2O_5 . This calculated to per cent. in the dry substance = about .75. Jordan (9) gives percentage in Timothy Hay .8, and in dry mixed grasses about .5; whilst the highest figure obtained for the grasses analysed in this laboratory = .41, so that we may take it that the introduced fodder grasses have adapted themselves just as the cereals have done¹ to the low-phosphorus soil in which they are placed, but that the adaptation is not complete since they still have a much higher percentage of P_2O_5 than the native grasses.

A (2).—Analyses of the wood of 4 typical Australian trees were also carried out. The trees in question were indigenous, and grew at Warrandyte, Victoria. A section taken right through a bough at the first bifurcation was ashed, and the P_2O_5 determined in the ash.

The following are the mean results of determinations for the per cent. P_2O_5 in the ash:—

¹ *Vide infra.*

<i>Eucalyptus amygdalina</i> or "Messmate" =	4.456 %
<i>Eucalyptus hemiphloia</i> or "White Box" =	2.436 %
<i>Eucalyptus haemostoma</i> or "White Gum" =	2.304 %
<i>Eucalyptus polyanthemus</i> or "Red Box" =	.856 %

Of these four the messmate is a very quick-growing tree, and is amongst the highest trees of the world. The Red Box, on the other hand, is a slow growing tree, with very hard wood. This difference very likely accounts for the diversity of the P_2O_5 content of the stems of these two, for wherever active growth is going on the P_2O_5 content is higher than in the inactive part, and the actively growing sap wood has always a higher percentage than the hard inactive heart wood.

Czapek (10) gives the percentage of P_2O_5 in the ash of the branch wood of *Pinus Silvestris* as 11.60; and the percentage in the heart-wood of a beech 220 years old 4.54, and in the sap-wood of the same 13.21; in the heart-wood of an oak 50 years old 5.88, and in the sap-wood 14.28; in the heart-wood of *Betula* 16.59, and in the sap-wood 13.21. All of these figures are higher than those obtained in the above analyses of the four Australian types taken, which fact again seems to point to an adaptation by plants to a phosphorus-low soil.

A further indication of adaptation is given by *Isopogon cera-
tophyllus*, a sample of which was kindly supplied by Dr. Cherry. This is a prickly shrub which only grows in poor soils, and on analysis was found to have an exceedingly low phosphorus content, viz.: 0.033% in the dried substance of the woody stems, and 1.41% in the ash of the same; and 0.046% in the dried substance of the leaves, and 2.09% in the ash of the leaves.

B.—EXPERIMENTS WITH SOME HUMAN FOODS.

The extraordinarily wide distribution of phosphorus in the body—in bones, nervous system, all the great gland organs, etc.—makes it an essential ingredient of food. According to Siven (1) the body needs at least .7 to .8 grams daily, and according to Ehrström more than this, viz., 1 to 2 grams daily. Hart, McCollum and Fuller (12) fixed 3 grams of phosphorus per day as the safe minimal quantity for a pig of 50 lbs. weight. The body has a greater power of retaining this

element than any other, in fact the body tries to hold back no other element as energetically as it does phosphorus. It deals with phosphorus in a more economic manner than with nitrogen, for with increased nitrogen intake the body immediately responds by increased nitrogen output to preserve the nitrogen equilibrium. With increased phosphorus intake, however, equilibrium is not established, but some of the phosphorus is retained (11). The amount of phosphorus actually absorbed from the alimentary canal depends on the kind of food—in vegetable foods which contain a large quantity of calcium a good deal of the phosphorus is left behind in the faeces as insoluble calcium phosphate; in meat feeding, on the contrary, most of the phosphorus in the faeces is due to actual intestinal secretion. In the food phosphorus is taken in for the most part in organic combination as nuclealbumen, nuclein, casein, lecithin, etc., in eggs, milk, leguminous vegetables, and only in small degree in inorganic combination as in grains, and meat.

The experiments of Hart, McCollum and Fuller at the Wisconsin Agricultural Experiment Station (12) show how important is a normal supply of P_2O_5 in food. Animals fed on food deficient in this constituent very soon became abnormal, exhibiting weakness of limbs, languor, debility, and if the experiment continued long enough, finally death; whilst if the diet was made normal again the animals gradually recovered. A disease, allied to rickets, is often found in cattle living on natural foods poor in calcium phosphate. In nearly all cases it is found if phosphates are added to the diet they are quickly absorbed with remedial effect; but a much better effect is gained by the addition to the diet of organic phosphorus combinations than by the addition of only the inorganic salts. The results of the experiments of Le Clerc and Cook (24) seem to point to the fact that organic phosphorus favours nitrogen metabolism, and increases nitrogen and phosphorus retention, especially in the case of a phosphorus poor food.

The researches of Cronheim and Müller (13) on children, and those of Röhmann, Ehrström and Gumpert (25), have shown organic phosphorus feeding to be superior to inorganic for growth and nourishment, and for this reason human milk is

said to be superior to cows' milk, which, though richer than human in total P_2O_5 , has yet a lower percentage of organic P_2O_5 ((14) Siegfried).

It is apparently a well-known fact that Australian cereals are lower in phosphorus content than the cereals of other countries; the general figure for wheat varies from 0.65 to 1.11 per cent. P_2O_5 in other countries, whereas 0.5 per cent. is taken as the average figure for Australian wheat; and likewise for oats; which fact points to an adaptation on the part of cereals to phosphorus-poor soils. From a physiological point of view it is the edible flour made from the wheat which is the most important. Experiments were therefore conducted with some local flour to determine whether the total per cent. P_2O_5 be low, and if so whether it be the organic or inorganic which is thus low, or both.

Method.—1. Total P_2O_5 determined in the flour weighed both dry and in the natural state. 2. Alcohol-soluble P_2O_5 determined by extracting the flour with boiling alcohol; evaporating extract, taking up residue with ether, and determining P_2O_5 in the ether residue. It is useless to try to extract with ether alone, for as early as 1891 it was shown by Maxwell (15) that ether would extract only the free lecithin, but would not take up any which was combined with protein.

The mean results obtained were:—

	Total P_2O_5	Alcohol Sol. P_2O_5	P_2O_5 not ex- tractable with alcohol
Per cent. in the undried flour	0.1932	0.0278	0.1654
Per cent. in the dried flour	0.2209	0.0322	0.1887

The results show the total P_2O_5 to be low, and the alcohol soluble moiety to be very small. Since bread is the mainstay and principal foodstuff, particularly of the poorer classes, and of vegetarians, the above fact is of physiological importance. It is very generally recognised by agriculturists that to obtain the best results possible the crops must be well manured by some kind of phosphate manure. If this is not done, and the crop has to depend entirely on the natural supply of phosphates, the entire yield is small, and individual grains poor.

It has therefore unfortunately to be admitted that Australian soils being low in Phosphorus cannot give of this element freely, with the result that plants grown here have to do with a small amount, and therefore consequently animals feeding on these plants obtain likewise a low supply. Remembering how widely distributed this element is in the body, and how conservative the body is of it, and also the results of Hart and McCollum on feeding animals with a low supply, it seems not unwarrantable to suggest the possibility of undesirable physiological features making their appearance as a consequence of this low percentage in one of our chief foods.

A few analyses of eggs were made. The eggs were all obtained from different local sources. The results obtained were:—

EGG 1.

Wt. with shell, grams	-	-	60.5696
Wt. edible part, undried, grams	-	-	54.3637
Wt. edible part, dried, grams	-	-	15.1069
Wt. ash of total edible part	-	-	0.5692
Per cent. water in undried edible part	-	-	72.2%
Total edible part contains, grams P_2O_5	-	-	0.327
Per cent. P_2O_5 in the undried	-	-	0.601%
Per cent. P_2O_5 in the dried	-	-	2.16%
Per cent. P_2O_5 in the ash	-	-	57.49%

EGG 2.

Wt. with shell, grams	-	-	59.7355
Wt. edible part, undried, grams	-	-	53.0364
Wt. edible part, dried, grams	-	-	14.0884
Wt. ash of total edible part	-	-	0.543
Per cent. H_2O in the undried edible part	-	-	73.4%
Total edible part contains P_2O_5 , grams	-	-	0.211
Per cent. in the undried	-	-	0.39%
Per cent. in the dried	-	-	1.500%
Per cent. in the ash	-	-	38.89%

EGG 3.

Total edible part contains P_2O_5 , grams	-	-	0.3307
Per cent. P_2O_5 in the dried	-	-	2.45%
Per cent. P_2O_5 in the egg shell	-	-	0.0160%

EGG 4.

Total edible part contains P_2O_5 , grams	-	0.3301
Per cent. P_2O_5 in the dry	-	2.51%
Per cent. P_2O_5 in the shell	-	0.0226%

EGG 5.

Wt. with shell, grams	-	52.757
Wt. edible part, undried, grams	-	45.584
Weight edible part, dried, -	-	12.534
Per cent. H_2O in undried edible part	-	72.5%
Total edible part egg contains, grams P_2O_5		0.245
Per cent. P_2O_5 in the undried	-	0.537%
Per cent. P_2O_5 in the dried	-	1.95%

Balland says (7) an ordinary hen's egg contains about .26 grams phosphorus.

Wolff's (16) analysis of hen's egg without shell gives 73.4 per cent. water, 3.48 per cent. total ash, and per cent. P_2O_5 in the ash 38.05, and in the dried 1.324.

Samples of cows' milk from three different sources were analysed for P_2O_5 content. Results obtained were:—

WILLSMERE MILK.

Sample 1	P_2O_5 in 100 c.c.	.	0.30354 grams
Sample 2	" " "	-	0.25458 "
Sample 1	Wt. of 5 "	-	5.0596 "
Sample 2	" " "	-	6.0354 "

TALBOT MILK.

Sample 1	P_2O_5 in 100 c.c.	-	0.21204 "
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PRIVATE DAIRY—KEW.

Sample 1	P_2O_5 in 100 c.c.	-	0.2040 "
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Bunge (17) says cows' milk contains .181 to .197 grams P_2O_5 in 100 c.c.

König (18) says cows' milk contains .189 grams P_2O_5 in 100 c.c.

Hutchinson (19) says cows' milk contains .220 grams P_2O_5 in 100 c.c.

It is to be noticed that in the case of eggs and milk the figures for the samples analysed here, are not lower than those given in English or German records. This fact is rather interesting, for both are secreted by the parent for the maintenance of the young, and the constituents of such secretions are nearly always kept in amounts such as are best suited for the development of the young; even at the expense of the mother and of the other secretions. When analysing the bones of frogs, it was found that the femur of a female frog, killed just before the breeding season, and containing a mass of small immature ova, contained a very high percentage of P_2O_5 , and it is suggested that this might be due to a storing of phosphate in the bone, which could be utilised when the time came for the development of the ova.

Experiments with Meat.—Results of analyses of fresh uncooked undried beef and mutton, practically fat-free, gave percentages of P_2O_5 the mean of which equalled about .38, and calculated to per cent. in the dried meat about 1.8 per cent., figures which agree with those of Francis and Trowbridge (20), but which are a little lower than those of Wolff.

Experiments with Fish.—Analyses of fresh uncooked fish gave mean per cent. P_2O_5 to be .51. This figure also accords well with other analyses. So that as far as the flesh foods go there is little variation in the local examples analysed from those analysed in other countries.

Again the figures for the oysters examined appeared to vary only slightly from the French and Portuguese (21) as far as percentage went, although the total P_2O_5 per oyster is very much smaller in the Australian than the others, owing to their small size. Thus:—

	Australian	French	Portuguese
Average % P_2O_5 in dried oyster	- 1.822	1.836	2.052
Average wt. of dried oyster	- 0.548	1.110	1.157
P_2O_5 contained in one average oyster	0.0097	0.020	0.032
Average % P_2O_5 in shell	- 0.0522	0.038	0.089

C.—EXPERIMENTS TO DETERMINE THE DISTRIBUTION AND EVOLUTIONARY SIGNIFICANCE OF PHOSPHORUS IN THE MUSCULAR TISSUE OF ANIMALS.

Determinations of the P_2O_5 percentage, in the muscular tissue of various animals, were made. In the case of the Mollusca the adductor muscles from a number of oysters were used for the experiments. In the case of the Arthropoda the body muscles were used. For the Vertebrates the muscles which serve to move the pelvic fin of fishes, and those which move the lower limbs of frogs, lizards, birds, dogs, etc., served as examples for Pisces, Amphibia, Reptilia, Aves, and Mammalia respectively.

The table shows the results:—

P_2O_5 IN MUSCLE—INVERTEBRATES AND VERTEBRATES.

Name	% P_2O_5 in fresh muscle	% P_2O_5 in dried muscle	% P_2O_5 in the ash	% ash in fresh	% ash in dried	Group	
Oyster, Australian (average of 33) - - -	.57	2.69	29.5	1.9	8.9	Mollusca	Invertebrates
Fresh-water "Yabbie" or Astacopsis - - -	.37	2.4	37.93	1.02	6.3	Arthropoda	
Mullet-male - - -	.51	2.22	38.1	1.3	5.8	Pisces	Vertebrates
Bream-female (no roe) -	.51	2.28	42.8	1.2	5.3	"	
Frog (average of adult female and young male)	.42	1.93	35.4	1.2	5.4	Amphibia	
Lizard - - - -	.41	1.90	36.0	1.2	5.3	Reptilia	
Parrot - - - -	.47	1.88	38.8	1.2	4.8	Aves	
Thrush - - - -	.43	1.75	34.5	1.2	5.0	"	
Mouse - - - -	.34	1.75	33.5	1.2	5.2	Mammalia	
Dog - - - -	.43	1.90	38.7	1.1	4.9	"	
Cow - - - -	.38	1.70	36.1	1.0	4.7	"	

It will be obvious from the above table that muscular tissue shows a surprising uniformity in its phosphorus content throughout the animal kingdom. Calculated in the dry substance the figures show the percentage to be slightly greater in the fishes and oysters than in the higher animals, but calculated in the ash the variations show no parallelism with degree of development. We may state therefore that phosphorus of this concentration, viz., .38 to .5, is apparently a normal and essential ingredient for contractile tissue.

D.—EXPERIMENTS TO DETERMINE DISTRIBUTION AND EVOLUTIONARY SIGNIFICANCE IN NERVE SUBSTANCE.

Analyses of brain substance of the various classes of Vertebrates were made. It is unfortunately impossible to get enough nerve tissue for analysis in the Invertebrates.

For results see table p. 383.

The experiments as far as they went show the brain substance in the examples of the various vertebrate classes examined to have practically the same percentage of P_2O_5 in the dried. Thus again in nerve substance there appears to be no parallelism between P_2O_5 content and evolutionary development.

E.—EXPERIMENTS TO DETERMINE THE DISTRIBUTION AND EVOLUTIONARY SIGNIFICANCE IN THE EXOSKELETON OF INVERTEBRATES.

Analyses of the exoskeletons of Invertebrates, comprising examples from the groups Porifera Coelenterata, Molluscoidea, Echinodermata, Arthropoda and Mollusca were made.

For results see table p. 383.

Name	Weight in undried	Weight dried	Weight dry	Weight as	Wt. P_2O_5 in amount taken	% P_2O_5 in fresh	% in the dried	% in the as	% ash in the fresh	% ash in the dried	Group
Fresh W. Murray Cod (mean of 4)	3.0721	0.43002		0.03612	0.01371	0.446	3.189	37.97	1.17	8.40	Pisces
Sea W. Barracouta (mean of 5)	6.11697	1.18767		0.09145	0.02716	0.444	2.286	29.69	1.49	7.70	
Frog (mean of 4)	1.3000	0.10977		0.0130	0.00353	0.270	2.29	27.15	1.00	11.1	Amphibia
Rooster (mean of 2)	4.9886	0.94798		0.06747	0.02099	0.420	2.21	31.33	1.33	7.11	Aves
Turkey	2.87619	0.55619		0.03851	0.01480	0.514	2.66	38.90	1.32	6.92	
Rabbit	6.2525	1.41454		0.09970	0.03149	0.503	2.26	31.58	1.59	7.05	Mammalia
Sheep	5.3299	1.00322		0.07354	0.02911	0.546	2.902	39.90	1.37	7.33	

P_2O_5 IN EXOSKELETON OF INVERTEBRATES.

Name	Weight taken undried	Weight dried	Weight ash	Wt. P_2O_5 in amount taken	% P_2O_5 in the undried	% P_2O_5 in the dried	% P_2O_5 in the ash	% ash in the undried	% ash in the dried	Group
Sponge	—	—	—	trace	trace	trace	trace	—	—	Porifera
Hydrozoa Zoophytes	—	—	—	trace	trace	trace	trace	—	—	
Coral 1	—	2.2902	1.70410	trace	trace	trace	trace	—	74.4	Coelenterata
Coral 2	—	2.7572	2.3497	trace	trace	trace	trace	—	85.2	
Polyzoon	—	0.10168	0.04420	trace	trace	trace	trace	—	43.47	Molluscoidea
Starfish 1	1.5950	1.29421	0.51882	0.00338	0.2121	0.261	0.615	34.40	42.4	
Starfish 2	2.1160	1.47725	0.70759	0.00453	0.2140	0.306	0.640	33.4	47.0	Echinodermata
Asciopsis or Fresh W. Yabbie 1	—	0.66523	0.28273	0.021102	—	3.17	7.14	—	42.5	
" " 2	—	1.4981	0.16831	0.04763	—	3.17	10.17	—	31.26	
" " 3	—	1.2293	0.38063	0.03431	—	2.79	9.51	—	29.33	Arthropoda
Sea W. Cray 1	—	1.49959	0.66553	0.01303	—	0.869	1.95	—	44.39	
" 2	—	1.7541	0.70695	0.02096	—	1.191	2.95	—	40.47	
Oyster 1	13.3948	42.1284	—	0.0184	0.0424	0.0136	—	—	—	
" 2	34.1714	33.13465	—	0.02017	0.0590	0.0608	—	—	—	
Cuttle-fish 1	1.4100	1.37314	0.8589	0.0023	0.1631	0.1670	0.265	60.92	62.5	Mollusca
" 2	1.9351	1.8834	1.30988	0.0034	0.1700	0.1800	0.258	67.69	69.5	

The results show several interesting features:—

1. Taking the class Invertebrata as a whole it will be apparent from the table that the protective or skeletal tissues derive their physical properties of rigidity, etc., from mineral matter other than phosphate, the latter only being present in small amount: the chief mineral salt being calcium carbonate. When a tissue is composed largely of calcium carbonate, this salt is invariably crystalline, and in consequence the tissue is brittle. Calcium phosphate on the other hand is capable of existing in a colloidal form, which does not render a tissue brittle. Apparently then the Invertebrates as a whole have not learned to avail themselves to any extent of colloidal calcium phosphate in the construction of their protective tissues; thus whilst they are rigid they are at the same time easily broken.

2. Taking the individual groups of the Invertebrata it is most interesting to note an increase in the P_2O_5 content of the exoskeleton as we ascend the evolutionary scale. Thus the Sponges, the Hydrozoa, the Corals, and the Polyzoa, have merely a trace of P_2O_5 as evidenced by a yellow coloration and a faint haze of precipitate with ammonium molybdate and nitric acid. Then the Starfish has a measurable quantity—.26–.30 per cent. In the group Arthropoda we find a marked rise, particularly in the fresh-water “yabbie” or *Astacopsis*, but not so marked in the salt-water crayfish, falling again to a low percentage in the oyster shell and cuttle fish of the next group, Mollusca. Some authors, however, would give the Arthropoda a higher place than the Mollusca, in evolution, and in regard to the utilisation of phosphorus in the exoskeleton they are certainly further on than Mollusca..

F.—EXPERIMENTS TO DETERMINE THE DISTRIBUTION AND EVOLUTIONARY SIGNIFICANCE OF PHOSPHORUS IN THE ENDOSKELETON OF THE VERTEBRATES.

In all the experiments in this section the bone analysed was the femur or that most comparable to the femur; thus in fishes the bone supporting the pelvic fin was the one used.

Results follow in tabular form:—

Name	Weight of fresh bone	Weight of dried bone	Weight of ash	Wt. P_2O_5 in bone taken	% P_2O_5 in the fresh	% P_2O_5 in dried bone	% in ash	% ash in the fresh bone	% ash in dried bone	Group
Shark 1 (female)	-	-	-	-	-	-	-	-	-	Pisces (Eusmbranchii)
" 2 (female)	-	-	-	-	-	-	-	-	-	
Fresh W. Murray Cod	-	-	-	-	-	-	-	-	-	
Sea W. Barracouta	-	-	-	-	-	-	-	-	-	Pisces (Teleostei)
Mullet 1 (male)	-	-	-	-	-	-	-	-	-	
" 2 (female)	-	-	-	-	-	-	-	-	-	
Perch 1 -	-	-	-	-	-	-	-	-	-	Amphibia
" 2 -	-	-	-	-	-	-	-	-	-	
Frog 1 (male)	-	-	-	-	-	-	-	-	-	
" 2 -	-	-	-	-	-	-	-	-	-	Reptilia
" 3 -	-	-	-	-	-	-	-	-	-	
" 4 -	-	-	-	-	-	-	-	-	-	
" 5 -	-	-	-	-	-	-	-	-	-	Avces
" 6 (female not breeding season)	-	-	-	-	-	-	-	-	-	
" 7 " early "	-	-	-	-	-	-	-	-	-	
Toad (male)	-	-	-	-	-	-	-	-	-	Mammalia
Turtle -	-	-	-	-	-	-	-	-	-	
Lizard -	-	-	-	-	-	-	-	-	-	
Parrot -	-	-	-	-	-	-	-	-	-	Mammalia
Thrush -	-	-	-	-	-	-	-	-	-	
Sparrow 1 (female)	-	-	-	-	-	-	-	-	-	
" 2 -	-	-	-	-	-	-	-	-	-	Mammalia
Echidna 1 -	-	-	-	-	-	-	-	-	-	
" 2 -	-	-	-	-	-	-	-	-	-	
Mouse 1 -	-	-	-	-	-	-	-	-	-	Mammalia
" 2 -	-	-	-	-	-	-	-	-	-	
Rabbit 1 (male)	-	-	-	-	-	-	-	-	-	
" 2 -	-	-	-	-	-	-	-	-	-	Mammalia
Cat (female)	-	-	-	-	-	-	-	-	-	
Dog (female)	-	-	-	-	-	-	-	-	-	
Sheep -	-	-	-	-	-	-	-	-	-	Mammalia
	-	-	-	-	-	-	-	-	-	
	-	-	-	-	-	-	-	-	-	

In this table, as in the preceding one, several interesting points should be noted:—

1. A marked difference between the magnitude of the P_2O_5 content of the skeleton of the Vertebrates and Invertebrates. Thus even in the shark where cartilage takes the place of true bone there is a sudden and unmistakable rise from the percentage in even the highest group of the Invertebrates; the metapterygium of the shark (a fairly young female) giving 32 per cent. P_2O_5 in the ash, and about 8.9 per cent. in the dried.

2. Tracing up the ascending groups of the Vertebrata, we find a marked rise from the cartilaginous to the bony fishes; but from there right on through amphibia, aves and mammalia, there is no very great variation, at any rate in the percentage value in the ash.

The cartilaginous fishes seem to stand in this respect midway between the main body of the Invertebrates on the one hand, and of the Vertebrates on the other—the Invertebrates, which have not yet learned the knack to any extent of utilising phosphorus in the skeleton, and the Vertebrates which have apparently early learned (even as early as the fishes) to utilise this element, and learned moreover to use it in amount best suited to the requirements, since from the fishes right on through the higher groups there is scarcely any variation; any small variations which do occur might easily be due to differences in food and environment. Although calcium phosphate is much superior to calcium carbonate, in that it can be kept much more readily in colloidal form than the carbonate can, and the characteristic rigidity and elasticity of bone can be maintained; yet this colloidal calcium phosphate is very unstable and constantly tends to slip into the crystalline form. The theory may be put forward that in the bones of animals this crystalline form is at once removed by the blood, and excreted as quickly as it is formed, and fresh colloidal phosphate takes its place, and thus is explained the necessity for metabolism in bone. In old age the replacement of the crystalline by colloidal is less efficient, and hence the bones become brittle.

G.—PHOSPHORUS IN AUSTRALIA FROM AN ECONOMIC VIEW POINT.

At the beginning of this paper it was pointed out that Australian soils as a whole are very low in phosphorus content, but

especially the superficial soils. Whether or no the total P_2O_5 in superficial soils plus sub-strata and sub-sub-strata, could we go deep enough, would be found to be lower than other countries it is hard to say, and the knowledge would be well nigh useless even were it possible. It is the available phosphorus which is the important thing, and if the available amount be small it matters little how large the inaccessible amount may be. In an extremely interesting paper (22) Dr. Cherry gives some possible causes for this deficiency. He points out the cycle by means of which inorganic rock phosphate fairly deep in the ground becomes converted into inorganic bone phosphate, and finally laid down superficially on the ground. The deep ground phosphate is dissolved by the acid juices of plant roots, absorbed and assimilated, becoming part of the plant substance. The plant will be either eaten by an animal and its phosphoric acid concentrated in the bony framework of the animal, which in the natural course of events will eventually die, and its skeleton after the organic matter has become oxidised be left deposited on the ground as superficial, easily-available phosphate; or else the plant untouched will also in time die, and a large amount of phosphorus stored in the leaves, fruit, bark and wood will be deposited superficially.

Since animals feed on plants there must of necessity be a continual struggle for survival, so that the larger and more numerous the animals are in any place, the greater will be the plant growth to keep pace, and consequently the roots will penetrate deeper into the earth and amongst other things a larger amount of phosphoric acid, hitherto deep buried and non-available, will become superficial and easily available. The low percentage of phosphoric acid in Australian soils is attributed by Dr. Cherry to the fact that Australia has never had any large land animals. Certainly there have been discovered near Lake Eyre, South Australia, areas of phosphate deposit, but these are probably due to crocodile fossils, and not to land animals.

Let us now view the state of affairs at the present day. Australia has opened out largely as a grazing country. This opening out has had two effects:—

1. The diminution in the number of native animals which were left in the olden times to die on the land and render up their phosphorus.

2. The introduction of a vast number of animals—viz., sheep, cattle, pigs, etc., which resemble the native animals of the olden time in that they convert plant-phosphorus into bone phosphorus, but which differ in this, that they are not left to die on the land, but to a large extent are exported, bones and all, right out of the country, carrying a large quantity of our much needed phosphorus.

In the past year Victoria alone is estimated to have carried some 12,500,000 sheep and lambs, besides cattle (23). If these sheep died on the land they would be helping on the cycle from deep to superficial phosphate. Of these 12,500,000 grazing, over three million are slaughtered, about 800,000 being exported, and the rest used for home consumption. The phosphorus in the faeces represents nearly all that which is returned to the land, only a small proportion of the sheep carried dying on the land except in a season of drought. The use of phosphatic manures has increased largely within the last few years, with advanced scientific knowledge and improved methods, but as yet it is largely the cultivated land which is benefited, and comparatively little the grazing land. It is interesting to get some approximate valuation of the phosphoric acid represented by 3,309,865 sheep—the number slaughtered. On an average the amount of phosphoric acid in the carcase of one sheep would be roughly 2.5 to 3 lbs. This gives an approximate estimate of 3699 tons of phosphoric acid taken from the grazing land by 3,309,865 sheep; of which about 892 tons are entirely lost to the State by the export of 800,000 sheep. A very considerable amount. The amount lost by beef is much less than by mutton; about 279,710 oxen being slaughtered for food per year, the average weight of a carcase being from 750 to 800 lbs., and amount H_3PO_4 in the carcase about 2 per cent., giving a loss of 1872 tons, about, from the grazing land; the amount entirely lost from the State in this way being only about 15 tons, since only 168,294 lbs. of beef are exported.

The Victorian Year Book, 1908-9 (23), shows the grand total number of pairs of frozen rabbits and hares exported oversea

from years 1902 to 1908, inclusive, to be no less than 25,416,445. These large figures suggested that this one export alone might be responsible for quite a considerable loss of phosphoric acid. Some typical export rabbits were secured; weighed fresh, dried in the oven at 100 degrees C., and then weighed again; the dried flesh separated from the bones and ground to a powder; the whole of it weighed, and an aliquot part taken and oxidised with boiling strong acids. The P_2O_5 precipitated as ammonium phospho-molybdate, redissolved, reprecipitated with magnesia mixture, filtered, washed, dried, ignited and weighed as pyrophosphate. The bones were treated in much the same way, dried and weighed. A little trouble was experienced in getting a homogeneous mixture, since the bones could not easily be ground to a powder as was the flesh. The difficulty was got over by wrapping them in a cloth to prevent any loss of flying particles, and crushing them with a heavy hammer so fine that the mixture could be taken as homogeneous. Results of experiments conducted in this way showed the amount of H_3PO_4 per total rabbit to be about 29 grammes, or 2.7 per cent. Taking the figures given above this makes an export of about 3.362.037 lbs., or 1500 tons of phosphoric acid in the rabbits exported during those seven years, or taking average for one year, 214 tons. Looked at in this light the rabbit appears not only a pest responsible for a good deal of damage to the pastoralist in an ordinary way, but also a pest which is constantly ridding land of valuable constituents. Of course, besides those used for export and home consumption, there are great numbers still left to die on the land; but these fail to be of much service by dying in their burrows, most of which are in low grazing land, and deep enough to be below the general level reached by plant roots. Having found that Victoria loses so much by her large export of mutton, and so much by an almost incidental export of a pest, we naturally turn to the large export—wool. Samples of export wool were obtained, weighed just as they were obtained; oxidised with acid, and the phosphoric acid determined in the same way as in the rabbit. The first experiments showed that the percentage of phosphoric acid in wool is low, and large

1 I here acknowledge my indebtedness to Dr. W. P. Norris for his kindness in securing samples of wool and export rabbits.

quantities of wool, therefore, had to be used in the estimations. Mean of experiments gave the percentage to be .0220. It is only the large amount of wool exported which makes the phosphoric acid lost in this way at all appreciable. For the year 1908-9 (23) the production of wool is given as 87,536,450 lbs., almost the whole of this being exported.

This gives the phosphoric acid lost in this way as 19.258 lbs., or about $8\frac{1}{2}$ tons.

From the cultivated land the chief loss is by wheat. In year 1908-9 (23) the total wheat production in Victoria was 23,000,000 bushels, about 2,000,000 bushels of which were returned to the land as seed, 21,000,000 bushels being thus lost to the cultivated land, and of those about 15,000,000 exported. The average percentage of phosphoric acid is about .5, in Australian wheat. The amount of phosphoric acid therefore lost to the cultivated land = 2929 tons and lost entirely to the State = 2097 tons.

Summarising these results:—

A.—Lost from Victorian Grass Lands per year.

				Lost from State.	
1.	By Mutton	-	-	3699 tons	- 892 tons
2.	By Rabbits	-	-	214 "	- 214 "
3.	By Beef	-	-	1872 "	- 15 "
4.	By Wool	-	-	$8\frac{1}{2}$ "	- $8\frac{1}{2}$ "
				<hr/> 5793 $\frac{1}{2}$ tons	<hr/> 1129 $\frac{1}{2}$ tons

B.—Lost from Cultivated Land.

				Lost from State.	
1.	By Wheat	-	-	2929 tons	- 2097 tons

When we remember this is in terms of pure phosphoric acid and not in terms of commercial manures (which usually only contain from 20-24 per cent. of H_3PO_4), and also in this list no account is taken of condensed milk, bacon, ham, cheese, etc., we cannot but realise the necessity for fertilising the land to make up for this continuous drainage. That this is made up to a very large extent on cultivated land is admitted, but the above figures show that the grass lands are submitted to a heavy leakage, and this has been going on, uninterrupted, more

or less steadily, ever since the country has been used for pasture; hence need to impress the necessity of returning to the grazing lands the essential element which they have been gradually losing for so long.

CONCLUSIONS.

1. The phosphorus content of the muscular tissue of all classes of animals is practically a constant.

2. The phosphorus content of the nervous tissue of all classes of Vertebrates is likewise practically a constant.

3. The phosphorus content of the exoskeleton of all Invertebrates is low, but is considerably greater in the higher groups than in the lower.

4. The phosphorus content of the endoskeleton of all Vertebrates is much higher than in the exoskeleton of Invertebrates, and varies very little in the different classes except that it is lower in the cartilaginous fishes.

5. Australian native grasses have a markedly lower phosphorus content than European.

6. Acclimatised European grasses have a higher phosphorus content than native Australian, but lower than the same kinds of grasses grown in Europe.

7. The wood of Australian trees has a lower phosphorus content than that of European trees.

8. The total phosphorus content of Victorian flour is low, and the alcohol soluble moiety particularly low.

9. The phosphorus content of Victorian eggs, milk and flesh foods does not vary appreciably from European.

10. The loss of phosphorus per year from Victorian grass lands, by export of their products, is considerable, and it is a matter of economic importance that the phosphorus thus abstracted should be returned.

I wish very much to express my indebtedness to Professor Osborne, in whose laboratory this research was conducted, and to thank him for his continued interest and assistance.

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